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Hypothermia induction and recovery in free-ranging rats

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Abstract

1. To avoid anesthesia confounders, free-ranging rats were exposed (4 h) to cool water (CW; 10 °C; 5 cm), warm water (WW; 35 °C; 5 cm) or temperate air (TA; 25 °C) to induce hypothermia, or control for water or novel environment stress, respectively.
2. While WW and TA core temperature (T_c) and the brown adipose tissue (T_{BAT})/subdermal skin (T_{SDS}) temperature relationship remained similar, CW hypothermia induction was variable (34.5–26.1 °C; 9–240 min) and associated with tachycardia (517.8±4.7 bpm) greater than WW (386.5±5.5 bpm) or TA (372.2±7.7 bpm) with CW T_{BAT} (36.5±0.03 °C) elevated above CW T_{SDS} (35.9±0.05 °C).
3. Without anesthesia to blunt thermoregulatory countermeasures to hypothermia, variable resistance to T_c depression, tachycardia rather than bradycardia and BAT thermogenic responses were demonstrated.

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Keywords: Hypothermia modeling; Accidental hypothermia; Hypothermia marker; Datalogger; Cold-induced tachycardia; Brown adipose tissue temperature

1. Introduction

Systemic hypothermia is commonly defined by a reduction in core temperature (T_c) below 35 °C (Biem et al., 2003; Rice, 2005). It is assumed that accidental hypothermia generally occurs at geographic latitudes supporting a winter season. However, hypothermia is both geographically and seasonally pervasive (MacDonell and Wrenn, 1991; Sim and Kuo, 2000; Danzl and Lloyd, 2001). Moreover, it is potentially lethal (Rice, 2005), as illustrated by the 16,555 hypothermia-related deaths from 1979 to 2002 in the United States (CDC, 2005). In the military setting, accidental hypothermia is of concern, since factors of military operational stress (exhaustive exercise, sleep deprivation and caloric restriction) may predispose to hypothermia (Young et al., 1998). Suitable animal models of hypothermia induction and recovery are required to study the predisposing factors and lethal effects of hypothermia. Unfortunately, hypothermia models are typically compromised by the use of anesthesia (Casey et al., 1983; Halvorson and Thornhill, 1993; Kader et al., 1992; Lee et al., 2001; Matthew et al., 2002; Holzer et al.., 2005; Martini et al., 2005; Kondratiev et al., 2006) or restrain (Shimada and Stit, 1983; Casey et al., 1983; Uchida et al., 1987). Anesthesia or restraint compromise normal thermoregulatory and behavioral mechanisms that function to maintain T_c homeostasis during exposure to environmental extremes. These confounders make such models less than idea for the study of hypothermia. The present report describes methods in free-ranging rats that avoid the use of anesthesia or restraint in hypothermia induction and recovery to provide a rodent model applicable to the study of accidental hypothermia.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (~60 g) were obtained from Harlan Inc. (Indianapolis, IN) and maintained under the NIH Guide for the Care and Use of Laboratory Animals

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and AAALAC. IACUC approved all study procedures. They were singly housed in polycarbonate cages (50 × 26.8 × 36.4 cm) with wood chip bedding (Pro-Chip, PWI Canada). In addition, running wheels to permit *ad libitum* voluntary exercise were provided as environmental enrichment (mean running distance just before experimentation = 3.9 ± 0.6 km/day with no significant differences among experimental groups). Rats were given rodent laboratory chow (Harland Teklad, LM-485; Madison WI) and water *ad libitum*, and maintained at 25 ± 2 °C on a 12 h light/dark cycle (0700 lights on). All rats were quarantined for 14 days before surgery and weighed daily to monitor heath status. Twice during this quarantine period, rats were provided with a marshmallow or *pina colada* treat impregnated in a maple wood chunk (Bioserv Product #F05475-1 and #W0002; Frenchtown, NJ) to provide environmental enrichment via support of foraging behavior.

2.2. Biotelemetry

Biotelemetry (Data Sciences International; St. Paul, MN) was employed to monitor T_c (±0.1 °C; $N = 12$/condition), motor activity (counts; $N = 12$/condition) and heart rate (beats/min [bpm]; $N = 6$/condition). Small mouse-sized transmitting devices (ETA-F20; 4.1 ± 0.01 g/1.9 cm^3) were employed to minimized potential negative influences of transmitter presence in the body cavity (Leon et al., 2004; Printz, 2004). These devices emitted a frequency proportional to T_c. The transmitters had leads extending from the transmitter body for the monitoring of heart rate. Before implantation and following experimentation, biotelemetry transmitters were calibrated to ensure measurement accuracy. Signals were collected at 1 min intervals by a receiver board placed beneath the home cage and/or experimental exposure tank (see below). Signals were converted to T_c using the pre-determined calibration values. Motor activity was detected by changes in signal strength as the animal moved over the receiver board. This was a general measure of activity, since it could not distinguish the type of locomotor action.

2.3. Dataloggers

Dataloggers (SubCue; Calgary, Canada) were used to record brown adipose tissue (BAT) or subdermal skin (SDS) temperature (T_{BAT} or T_{SDS}, respectively; $N = 12$/condition). Dataloggers are 1.5 cm diameter and 0.5 cm thick cylinders weighing 4.1 ± 0.02 g with a temperature sensing (±0.1 °C) element in the center of the device. These devices do not provide real time viewing of the data, since they require removal from the animal for down loading of the stored data. Before implantation, a correction factor for each datalogger was determined over a temperature range of 30–38 °C and the dataloggers programmed to initiate data collection for 1.4 days at a 1 min intervals starting at 07:00 on the day of experimentation. After data down loading, the correction factor was applied to adjust the datalogger values.

2.4. Surgical procedures

Surgical procedures were initiated following the 14-day quarantine period. Rats were anesthetized with isoflurane gas. Transmitters were implanted by suturing them to the interior abdominal wall just caudal to the sternum to ensure the exterior abdominal wall covering the transmitter would not be exposed to water during experimentation. Transmitter heart rate leads were positioned on the upper right side and caudal to the left side of the rib cage. Dataloggers were implanted beneath the dermal skin layer over the subscapular BAT or just caudal to this BAT deposit for measurement of T_{BAT} or T_{SDS}. To minimize shift in datalogger position, dataloggers were sutured to the musculature beneath the dermal skin layer. Immediately following surgery, rats were provided intraperitoneally indomethacin (1 mg/kg) in 2 hydroxypropyl β-cyclodextrin (Sigma, St. Louis, MO) and ampicillin/polyflex® (100 mg/kg; Fort Dodge, IA). Needle gauge for indomethacin and ampicillin injection was 26 and 23, respectively. The following day, rats were given for voluntary consumption a pina colada oral treat (BioServ; Frenchtown, NJ) impregnated with an additional dose of indomethacin. Each day post-surgery, rats were weighed to monitor recovery.

2.5. Experimental procedures

Fourteen days post-surgery, when pre-surgical body weight (169.6 ± 2.7 g) was exceeded (250.7 ± 5.1 g) and a robust circadian rhythm for T_c and motor activity established, rats ($N = 12$/condition) were exposed to cool water (CW), warm water (WW) or temperate air (TA) conditions. The Plexiglas® exposure tanks were similar to housing cages in dimension (43.5 × 24.7 × 30.6 cm) and like housing cages had two water sipper openings (1.9 cm diameter; 8.3 cm from tank bottom) on opposite sides of the tank that were clear of obstruction. Positioned just below the water sipper openings were small shelves. Unlike the TA tank, the CW and WW tanks (Fig. 1) were attached to a circulating water bath (Thermo Electron; Newington, NH) by a port from which water was delivered (0.7 ± 0.3 L/min) into the tank. Diagonal to the water entry port was a siphon tube that was connected to the return vacuum of the water bath. Water depth was regulated by adjusting the distance of the end of the siphon tube from the tank bottom. To accustom rats to the sounds of water bath operation, each day for 2 weeks before experimentation, water baths were engaged during that portion of the day in which experiments were to be conducted. Time 0 (baseline between 09:00 and 11:30 h) was defined by a 12 h average T_c (37.27 ± 0.02 °C) and activity (1.51 ± 0.16) nadir for Sprague–Dawley rats under the environmental conditions of the animal facility. At time 0, a rat was placed in each of three tanks that remained uncovered and exposed to

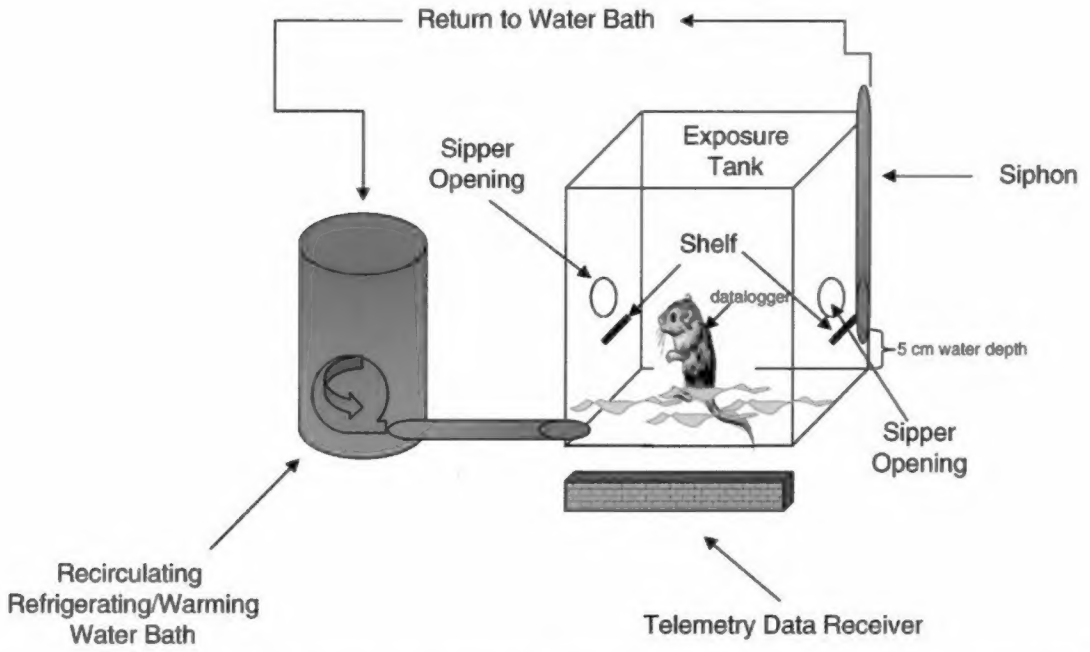

Fig. 1. Schematic of the exposure tank for cool or warm water exposure in which rats typically assumed an upright posture such that only their lower hindquarters and tail were immersed in water. Position of brown adipose tissue datalogger and telemetry data receiver is also depicted. Tanks for rats exposed to temperate air were similar in design with the exception the tank remained dry.

ambient air (25±2 °C). The TA tank was dry and maintained at this ambient air temperature. The WW tank contained 5 cm of circulating 35 °C water. Water depth in the CW tank was also 5 cm, but water temperature was maintained at 10 °C, which served as the driving force to reduce rat T_c. Rats remained under these conditions for a period of 4 h (240 min). After which, they were removed from their respective exposure environments and returned to their home cages to recover without handling for 3 days at 25±2 °C.

2.6. Hypothermia induction and recovery markers

To describe a bout of hypothermia and recovery, markers as illustrated in Fig. 2a,b were defined as follows:

Hypothermia depth = lowest T_c obtained over the 240 min exposure period.
Induction time (min) = length of time to lowest T_c.
Cooling rate (°C/min) = T_c at Time 0−lowest T_c/induction time.
Thermoregulatory maintenance time (min) = 240 min−induction time.
Recovery time = time required for T_c to return to 37 °C−240 min.
Rewarming rate (°C/min) = 37 °C−T_c at 240 min/recovery time.
Maximum T_{BAT} (°C) = highest T_{BAT} recorded during 240 min exposure period.
BAT thermogenic response time (min) = length of time CW exposure $T_{BAT} > T_{BAT}$ pre-exposure (−120 to 0 min) mean.

2.7. Data analysis

Data are presented as means ±SEM. T_c is shown as either individual rat 1 min values or group means for CW, WW and/or TA conditions. Sigma Stat 3.0 (Systat Software, Richmond, CA) was used in the data analysis. Two-way ANOVA with repeated measures followed by Holm–Sidak post hoc testing was employed to determine significant differences in T_c, T_{BAT}, T_{SDS}, heart rate and activity profiles between or among groups. Spearman rank order determined correlation coefficients for markers of hypothermia induction and recovery. Significance was set at $p<0.05$.

3. Results

3.1. Rat activity (Fig. 3) and behavior with CW, WW or TA exposure

Before and shortly after the CW, WW or TA exposures, rat activity was similar under each condition. For a short period (23 min) after initial exposure, TA rats had significantly higher activity (Fig. 3) than CW and/or WW rats. This activity was presumably due to the exploration of their new exposure environment, after which TA rats tended to settle into a curled position representative of a state of rest or sleep. Generally, when first placed in the CW or WW exposure tank, rats exposed only their feet and tail to the water. One CW rat attempted to extricate itself from the tank by jumping, which resulted in its splashing in the water to totally immerse its coat before positioning

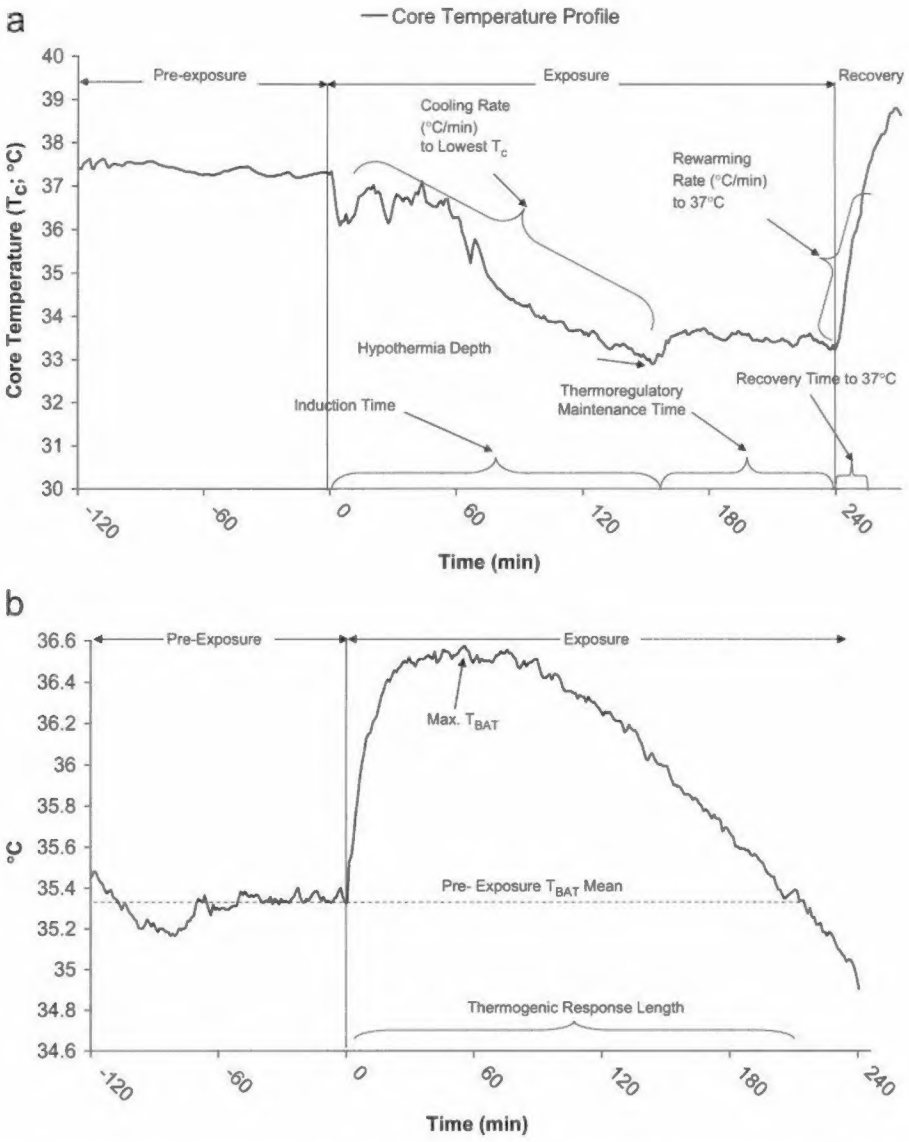

Fig. 2. Illustration of hypothermia induction and recovery markers: (a). Markers derived from core temperature profile pre, during and post cool water exposure. (b). Markers derived from brown adipose tissue (BAT) temperature (T_{BAT}) profile pre, and during cool water exposure.

itself at the sipper opening. All other CW and WW rats quickly positioned themselves at the sipper opening. They rested their forepaws on the small shelf below and assumed an upright body posture such that only their tails and lower hindquarters were exposed to the water (Fig. 1). They remained at the sipper opening with little or no movement during CW or WW exposure. As such, the wetted area of water-exposed rats was generally quite similar and limited to the lower hindquarters and tail. Following removal from the exposure tanks and placement back into their home cages, TA rats once again demonstrated higher activity (see 243 and 279 min time segment) than CW and/or WW rats. All groups had increased activity approaching the 600 min time point.

3.2. T_c changes with CW, WW or TA exposure (Fig. 4)

Before exposure, no significant differences in T_c (Fig. 4) for CW (37.4 ± 0.01), WW (37.4 ± 0.02) or TA (37.4 ± 0.03) rats were noted. With exposure, hyperthermia developed in WW (37.8 ± 0.05) and TA (37.9 ± 0.06) rats that slowly waned throughout the 240 min exposure. During exposure no significant differences in T_c between WW and TA rats were noted. At the point of lowest T_c reduction in CW rats, T_c was approximately 6 °C below that observed in WW and TA rats (31.6 ± 0.8 °, 37.5 ± 0.06 and 37.5 ± 0.12 °C, respectively). Moreover, the mean CW T_c thermoregulatory curve was significantly depressed compared to WW and TA throughout the 240 min exposure period. As illustrated

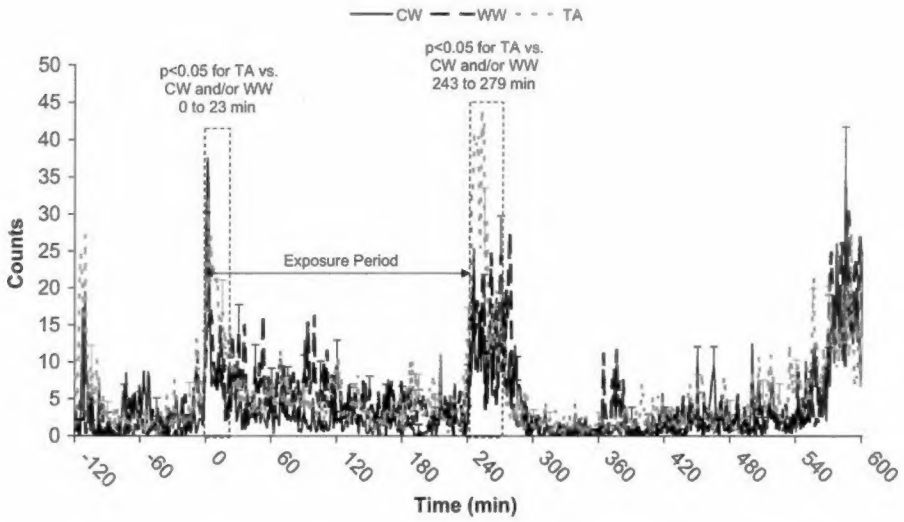

Fig. 3. Rat activity comparisons among cool water (CW), warm water (WW) and temperate air (TA) exposure ($N = 12$/condition).

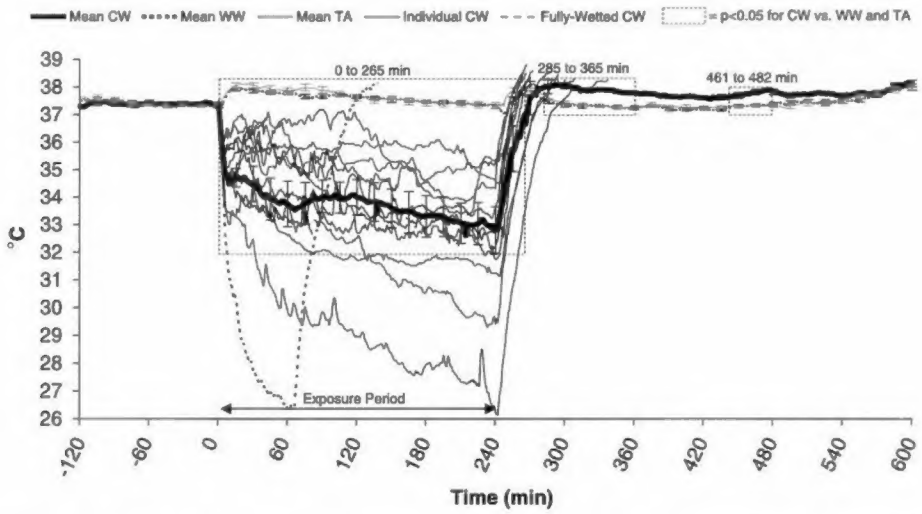

Fig. 4. Rat core temperature for individual, fully wetted and mean cool water (CW) compared to mean warm water (WW) and mean temperate air (TA) exposure conditions ($N = 12$/condition).

in Fig. 4, considerable variability in the individual T_c profile was displayed among the 12 CW rats. Also shown is the effect on the rat that immersed its coat with the cool water as a result of its jumping in the tank. This rat's exposure to the CW condition was terminated at 60 min, because of its rapid, precipitous and continuous cooling (0.2 °C/min) compared to other animals (0.05 ± 0.02 °C/min). With removal from the CW exposure tank, rats experienced a steep increase in T_c slope as T_c returned to 37 °C. Following removal from the exposure tanks, hyperthermia was experienced by CW (38.0 ± 0.01 °C), WW (37.7 ± 0.02 °C) and TA (37.9 ± 0.04 °C) rats. This soon dissipated for WW and TA rats, but lingered for CW rats such that CW T_c was significantly elevated above WW and TA rats for the time segments of 285–365 and 461–482 min post-exposure.

3.3. BAT and SDS temperature (Fig. 5a and b)

For all rats, the T_{BAT}/T_{SDS} relationship before exposure was one in which T_{BAT} (35.5 ± 0.05 °C) ran significantly below T_{SDS} (36.0 ± 0.03 °C). However, during CW exposure, this relationship reversed such that T_{BAT} ran above T_{SDS} and became significantly greater (36.5 ± 0.03 vs. 35.9 ± 0.05 °C) over the 9–115 min time segment (Fig. 5a). The T_{BAT}/T_{SDS} relation noted before exposure largely remained similar during TA exposure (data not shown). With WW, T_{BAT} increased such that significant differences relative T_{SDS} noted before exposure were now no longer apparent (data not shown). Following CW exposure, the T_{BAT}/T_{SDS} relationship reverted back to that noted before exposure in which T_{BAT} (35.8 ± 0.08 °C) ran significantly below (Fig. 5a, 310–598 time segment) that of T_{SDS}

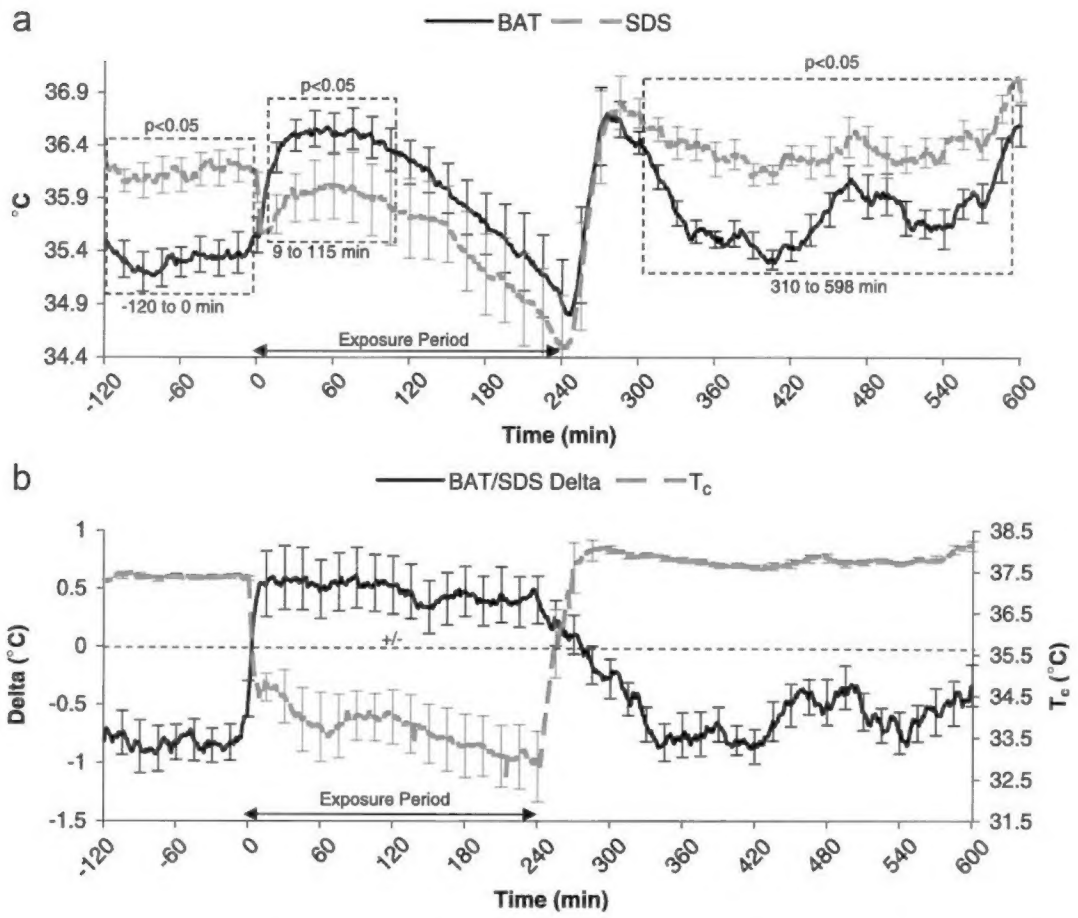

Fig. 5. a. Comparison between rat brown adipose tissue (BAT; $N = 12$) and subdermal skin (SDS; $N = 12$) temperature responses with cool water exposure. b. Comparison between rat brown adipose tissue (BAT)/subdermal skin (SDS) delta and core temperature (T_c) during cool wet exposure ($N = 12$).

Table 1
Markers of hypothermia induction and recovery

	Core temperature (T_c; °C) at hypothermia depth	Induction time (min) to hypothermia depth	Thermoregulatory maintenance time (min)	Cooling rate (°C/min)	Maximum brown adipose tissue (BAT) temperature (T_{BAT}; °C)	T_{BAT} Thermogenic response length (min)	Hypothermia recovery time (min) to a $T_c = 37$ °C	Rewarming Rate (°C/min)
Range	26.3–34.5	9–240	0–231	0.01–0.3	36.1–37.4	12–240	10–47	0.11–0.29
Mean	31.6	185.3	39.7	0.06	36.7	174.8	24.3	0.22
SEM	0.8	22.1	20.0	0.03	0.1	21.8	4.2	0.02

Values are means ± S.E.M

(36.4 ± 0.05 °C). Similarly, following WW or TA exposure, the T_{BAT}/T_{SDS} relationship assumed a pattern as seen before exposure (data not shown). As illustrated in Fig. 5b, a positive T_{BAT}/T_{SDS} delta was experienced during the decrease in T_c associated with the CW exposure. Moreover, a positive BAT thermogenic response relative to the pre-exposure (−120–0 min) mean T_{BAT} was noted for much of the 240 min exposure period (Table 1).

3.4. Heart rate changes with CW, WW or TA exposure (Fig. 6)

Before exposure, no significant differences in heart rate were noted among CW (341.3 ± 5.3 bpm), WW (338.6 ± 6.1 bpm) and TA (337.0 ± 6.4 bpm) designated rats. During exposure, CW (535.5 ± 1.1 bpm), WW (428.7 ± 5.7 bpm) and TA (427.3 ± 5.0 bpm) rats developed significant

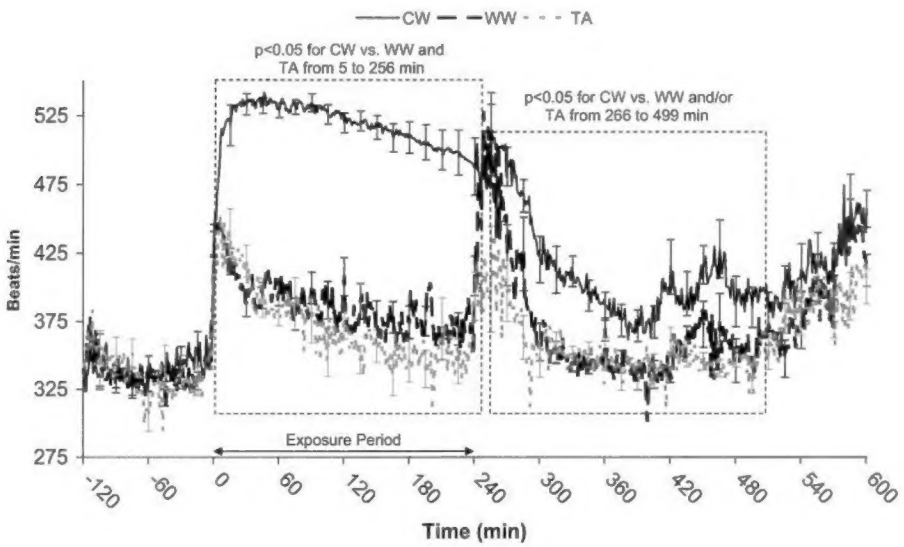

Fig. 6. Rat heart rate comparisons among cool water (CW), warm water (WW) and temperate air (TA) exposure ($N = 6$/condition).

tachycardia that reached a maximum within the first hour of exposure. As illustrated in Fig. 6, tachycardia tended to dissipate over the 240 min exposure, however tachycardia associated with CW (517.3 ± 6.2 bpm) remained significantly elevated compared to WW (391.3 ± 12.0 bpm) and TA (373.1 ± 10.8 bpm) throughout the exposure period and for a short time thereafter (5–256 min). During recovery from CW exposure, heart rate (406.6 ± 12.3 bpm) remained significantly elevated relative to WW (358.1 ± 10.5) and/or TA (350.1 ± 6.1 bpm) from the 266 to the 499 min time segment. All groups showed increasing heart rates as the 600 min time point approached.

3.5. Markers of hypothermia induction and recovery with correlations (Tables 1,2)

A number of markers were defined to characterize a bout of hypothermia (Table 1). These markers described not only the depth of hypothermia (i.e., lowest T_c obtained), but provided objective measures of the capacity of thermoregulatory countermeasures to resist hypothermia induction (i.e., time to reach the lowest T_c; thermoregulatory maintenance time; cooling rate; BAT thermogenic response length; maximum T_{BAT}) and the thermoregulatory recovery capacity to return to a normal T_c (i.e., time to return to 37 °C; rewarming rate). Table 2 shows which markers had significant positive or negative correlation coefficients. These correlations demonstrated that BAT function supported thermoregulatory maintenance, which influenced the time and the T_c at hypothermia depth. They also revealed the inverse relationship between hypothermia depth and recovery time.

4. Discussion

4.1. Controls for inherit confounders

In the absence of anesthesia, animals are fully cognizant, a hypothermia model that employs water to reduce body temperature must account for the psychological stress and subsequent physiological responses endured by an animal naive to placement in a body of water. Repeated bouts of 35 °C water exposure over 4 days showed no significant diminution in the hyperthermic responses to suggest that rats were unlikely to be accustomed to water exposure (data not shown). As such, water stress could be a major confounder of thermoregulatory responses under the present design. Another potential confounder was rat removal from the home cage and subjection to the novel tank environment. To account for the contribution of these confounders, rats exposed to WW or TA conditions were employed as experimental controls. Using a water temperature (35 °C) in which WW rats had similar T_c responses as TA rats (Fig. 4), the WW condition delineated the contributions of water stress, while responses under the TA condition captured the stress associated with exposure to the novel tank environment. As such WW and TA controls could be used to factor-out confounders to identify a feature unique or the portion of a response attributable to the actions of body cooling.

4.2. Rat activity and behavior with CW, WW or TA exposure

Rat activity under these various exposure conditions showed few significant differences, with the exception of TA, which was significantly elevated compared to CW

Table 2
Correlation coefficients for hypothermia induction and recovery markers in which $p<0.05$

	Induction time (min) to hypothermia depth	Thermoregulatory maintenance time	Maximum brown adipose tissue (BAT) temperature (T_{BAT})	BAT thermogenic response length	Hypothermia recovery time to $T_c = 37\,^{\circ}C$
Core temperature (T_c; °C) at hypothermia depth	−0.589	+0.899	+0.590		−0.889
Induction time to hypothermia depth		−0.713			
Thermoregulatory maintenance time			+0.634	+0.588	−0.856

Paired variables with positive correlation coefficients tended to increase together. Negative correlation coefficients indicated one variable increased, while the other decreased.

and/or WW shortly after exposure initiation and again following the removal from the exposure tanks (Fig. 3). The differences at exposure initiation likely reflected the tendency of water-exposed animals to keep as much of their body out of the water as possible. As such, they quickly found the sipper opening and the shelf below where they positioned their forepaws to sustain an upright posture in which only their lower hindquarters and tail where exposed to the water (Fig. 1). Rats rapidly learned any movement away from the shelf enhanced, the potential of slipping from the tank wall to expose more of their body to the water. Thus, water-exposed rats remained relatively stationary at the sipper opening, such that wetted area among rats was very similar. In contrast, TA rats tended to explore the novel tank environment for a longer time period before achieving what appeared to be a state of rest or sleep. Immediately following exposure, the elevated activity by TA relative to CW and/or WW rats perhaps resulted, because water-exposed rats appeared to exert less activity as they sat grooming themselves to remove the wetness from their coats. In any case, activity differences seemed inconsequential, since they did not result in significant T_c differences in TA and WW rats (Fig. 3). Increases in rat activity as the 600 min time point approached likely reflected anticipation of the lights-off period in which nocturnal animals normally become more active. This perhaps also accounted for the upswing in T_c (Fig. 4) and HR (Fig. 6) around this time.

4.3. Hypothermia induction/recovery and countermeasure action

Rat exposure to 5 cm of circulating 10 °C water induced a state of mild to moderate hypothermia in a workable timeframe of 4 h in which T_c was significantly less than rats exposed to the WW and TA conditions (Fig. 4). This temperature reduction developed under the full effects of behavioral (e.g. body posture to limit water exposure) and thermoregulatory countermeasures (e.g. shivering, vasoconstriction, BAT thermogenesis, etc.) attempting to sustain a normal T_c that would be thwarted by the presence of anesthesia (Kilgour and Williams, 1996; Maggi and Meli, 1986; Strobel and Wollman, 1969; Ohlson et al., 2003; Kiyatkin and Brown, 2005) or restraint (Shimada and Stit, 1983). Since with one exception rat-wetted areas appeared similar, differences in countermeasure effectiveness perhaps contributed to the broad range in marker values for hypothermia induction and recovery (Table 1). Thermoregulatory maintenance time and maximum T_{BAT} had significant positive correlation coefficients with hypothermia depth, while thermoregulatory maintenance time correlated negatively with hypothermia induction time (Table 2). As such, the broad range in thermoregulatory maintenance time and maximum T_{BAT} values (Table 1) suggested differences in the intensity of these countermeasure actions contributed to the variability in hypothermia induction. In anesthetized animals where countermeasures are essential blocked, cooling variability would likely not be as great, which is a primary example of anesthesia's presence compromising hypothermia modeling.

As rapidly as hypothermia was induced, recovery following removal from the CW condition was equally rapid, as illustrated by the steep, positive slope in the recovery T_c (Fig. 4). A wide recovery time range was explained by the wide range in hypothermia depth (Table 1) and confirmed by the significant negative correlation coefficient between hypothermia depth and hypothermia recovery time to 37 °C (Table 2). The significant negative correlation between recovery time and thermoregulatory maintenance time was a consequence of the significant positive correlation between the T_c at hypothermia depth and thermoregulatory maintenance (Table 2).

The factors influencing the periods of significantly elevated T_c in CW rats following recovery (Fig. 4) was not determined. However, enhanced activity post-CW exposure did not contribute to this finding, since no significant differences were noted during these time periods (Fig. 3). Though unobserved behavioral contributions (e.g. nesting differences among groups) could not be excluded, the prolonged tachycardia noted post-CW exposure (Fig. 6) in conjunction with the possibility of an increased metabolic rate was one probable explanation for these periods of significant T_c elevation. Such elevations were the only post exposure T_c differences noted, since over the next 3 days T_c was similar for previously exposed CW, WW and TA rats (data not shown).

4.4. Datalogger BAT monitoring

While primate (Casey et al., 1983) and swine (Holzer et al., 2005; Martini et al., 2005) hypothermia models are reported, most hypothermia models employ small rodents, mainly the rat (Halvorson and Thornhill, 1993; Kader et al., 1992; Lee et al., 2001; Matthew et al., 2002; Kondratiev et al., 2006; Shimada and Stit, 1983; Uchida et al., 1987). When using the rat in hypothermia modeling, BAT monitoring is an important parameter, since this specialized thermogenic tissue in which ATP production is uncoupled to generate heat plays a significant thermoregulatory role in small mammals (Cannon and Nedergaard, 2004). In the present study, dataloggers placed over the subscapular BAT deposit were used to capture the thermogenic response of this tissue. That BAT relative to SDS dataloggers registered significantly elevated temperatures and a positive delta (Figs. 5a, b), demonstrated dataloggers could discriminate temperature differences from sites at or away from BAT deposits. Moreover, the significant positive correlation of BAT thermogenic response length and maximum T_{BAT} with rat thermoregulatory maintenance time (Table 2), illustrated the usefulness of dataloggers to identify BAT function as an important countermeasure resisting hypothermia induction.

The use of dataloggers resolved, in part a limitation of standard telemetry in which temperature measurement at multiple sites is not available. However, the inability of dataloggers to support discrete points of temperature measurement because of their large size and thickness meant these devices could not be readily adapted to obtain tail temperature. This is another import parameter, since the tail is a major thermoregulatory organ in rats. The design of dataloggers with an exteriorized sensing element leading from the data storage body would allow for discrete tail temperature measurements to augment studies employing standard telemetry in rats. Moreover, discrete point measurement by dataloggers would improve specificity and sensitivity of T_{BAT}/T_{SDS} determinations.

4.5. CW exposure-related tachycardia in free-ranging rats

Significant tachycardia was associated with CW exposure that continued long into the recovery period (Fig. 6). This finding in free-ranging animals contrasted with reports from rats anesthetized before hypothermia induction in which significant bradycardia characterizes the cardiac response throughout hypothermia induction and recovery (Kondratiev et al., 2006). Bradycardia is also a feature in anesthetized swine at a hypothermic T_c of 33 °C (Holzer et al., 2005) in which tachycardia would generally be expected (Biem at al., 2003). In accidental hypothermia, tachycardia coupled with increased metabolism functions to defend body temperature during a cold challenge. However, with prolonged cold exposure as energy is depleted and body cooling deepens, bradycardia begins to characterize cardiac function. Thus, there is a transition from tachycardia to bradycardia (Biem et al., 2003), not seen in animal models employing anesthesia (Kondratiev et al., 2006). As such, cardiovascular interpretations from such models may not adequately reflect accidental hypothermia. As noted above in regards to hypothermic T_c response variability (Fig. 4) likely stemming from thermoregulatory countermeasure effectiveness in free-ranging rats, the presence of tachycardia in mild to moderate hypothermia further exemplified the enhanced relevance to accidental hypothermia of an animal model avoiding anesthesia.

5. Conclusions

The present study demonstrated the feasibility of hypothermia induction and recovery in free-ranging rats exposed to circulating cool water, as a model of accidental hypothermia. In the un-anesthetized state, the behavioral and physiological countermeasures mobilized to resist a fall in T_c were experienced. Thus, the full milieu of physiological inputs appropriately influenced all study parameters during hypothermia induction and recovery. This strengthened the relationship of the findings to accidental hypothermia. Rats generally tolerated the CW exposure, with the exception in one case in which an initial, short period of rat agitation cause the animal to become fully wetted by the cool water to obtain a $T_c < 27$ °C within the first hour of exposure (Fig. 4). This suggested profound hypothermia in the free-ranging state could be achieved by increasing water depth and/or decreasing temperature to enhance further the applicability of this model in hypothermia studies. Finally, markers (Table 1) and their correlations to hypothermia induction and recovery (Table 2) were identified to characterize a bout of hypothermia. Employing such markers, the influence of pre-disposing factors (drugs, alcohol, exhaustion, sleep deprivation, etc.) on hypothermia could be assessed. The present design employing free-ranging animals could contribute to such assessments.

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In conducting the research described in this report, the investigators adhered to the "Guide for Care and Use of Laboratory Animals" as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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